Expression of an Epitope as Detected by the Novel Monoclonal Antibody 4F7 on Dermal and Epidermal Dendritic Cells. I. Identification and Characterization of the 4F7$^+$ Dendritic Cell *In Situ*

Mansour Mohamadzadeh,* Thilo Lipkow,* Gerhard Kolde,† and Jürgen Knop*
Department of Dermatology, Universities of *Mainz, and †Münster, Germany

Ears of Balb/c mice were treated epicutaneously with 0.5% 2,4-dinitrofluorobenzene (DNFB) to obtain monoclonal antibodies characterizing molecules on epidermal dendritic cells that are involved in the induction and elicitation of allergic contact dermatitis. Six hours after this treatment, epidermal cells were prepared from the ear skin, and Ia-positive cells were enriched by indirect panning and injected into rats.

Hybridomas were generated and supernatants were screened for antibodies on ear skin from DNFB-treated and untreated animals. A clone (4F7) was isolated and characterized by immunohistochemistry and immunoelectron microscopy on murine skin and other organs. The monoclonal antibody 4F7 (IgG_1) recognized distinct dendritic cells in the dermis and very few dendritic cells in the paracortical area of the lymph nodes, the white pulp of the spleen, and the mucosa of the large intestine in normal animals. By fluorescence activated cell sorter analysis, it stained about 1.64% of the dermal and no epidermal cells in the skin of untreated animals. Approximately 50% of the dermal 4F7$^+$ cells expressed Ia molecules on their surface.

Six hours after application of 0.5% DNFB, the expression of the 4F7 antigen was strongly enhanced *in vivo* on dendritic cells in both the dermis and epidermis. About 15% of the epidermal dendritic cells expressing 4F7 exhibited Birbeck granules, the other Birbeck granule-negative cells resembled indeterminate dendritic cells (IDCs).

The dermal and epidermal 4F7$^+$ cells could be highly (98%) enriched with 4F7-labeled immunomagnetic particles. Transmission electron microscopic analysis of such preparations showed typical characteristics of dendritic cells with 50% or 100%, respectively, of these cells expressing Ia molecules on their cell membrane. The results suggest that the 4F7 epitope is expressed on dendritic cells related to Langerhans cells and is upregulated by an inflammatory stimulus. Key words: allergic contact dermatitis/DNFB/4F7$^+$ cells. *J Invest Dermatol 101:832–838, 1993*

Allergic contact dermatitis is mediated by primed T cells that induce a specific inflammation after contact with hapten [1]. Activation of the antigen-specific T lymphocyte requires presentation of the antigen in association with major histocompatibility complex (MHC) class II antigen on antigen-presenting cells (APCs) [1].

Additional signals are provided by factors such as interleukin (IL) 1 and 2, adhesion molecules [2], and other co-stimulatory signals, which are not clearly defined. Some of these stimulatory activities may be induced by contact sensitizers in APCs or other accessory cells such as macrophages, keratinocytes, or endothelial cells. It has been shown that contact sensitizers induce in Langerhans cells (LCs) receptor-mediated endocytosis [3,4], loss of membrane adenosine-5′-triphosphate (ATP)ase activity [5], and possibly migration of the cells into the draining lymph node [6]. This indicates that contact sensitizers are able to activate mechanisms in LCs that may be relevant for sensitization. The nature of these costimulatory mechanisms and the molecules involved are not clearly understood.

To identify molecules that are induced on Ia$^+$ epidermal cells shortly after application of a contact sensitizer onto the skin of non-sensitized mice, we generated monoclonal antibodies (MoAbs) to epidermal cells enriched for Ia$^+$ dendritic cells from mice treated with the contact sensitizer 2,4-dinitrofluorobenzene (DNFB). A MoAb was isolated that reacted with an epitope on Ia$^+$ dermal dendritic cells (DCs) of DNFB-untreated mice. This epitope was upregulated and also found on epidermal DCs, some of which resembled LCs, after DNFB application.

Manuscript received May 26, 1992; accepted for publication July 10, 1993.

Reprint requests to: Prof. Dr. med. J. Knop, Hautklinik der Johannes Gutenberg, Universität, Langenbeckstrasse 1, D-6500 Mainz, Germany.

Abbreviations: AEC, 3-amino-9-ethylcarbazole; IDC, indeterminate dendritic cell; PEG, polyethylene glycol.

Part of this work was presented at the 21st Annual Meeting of the ESDR, Copenhagen, 1991.

MATERIALS AND METHODS

Animals Balb/c mice were purchased from the Zentralinstitut für Versuchstierkunde in Hanover, Germany. For our experiments we used female mice at an age of 8–12 weeks and DA/HA rats from the same institute about 8 weeks old for immunization.

Culture Medium RPMI 1640 (Biochrom KG, Berlin, FRG) supplemented with 10% fetal calf serum and 20 μg/ml gentamycin (both Biochrom KG) was used.

0022-202X/93/$06.00

Antibodies MKD6 (anti-I-A^d [7]) was obtained from American Type Culture Collection (ATCC, Rockville, MD), goat–anti-rat polyclonal antibody conjugated with beads from Dianova (Hamburg, Germany), and sheep–anti-rat peroxidase-conjugated polyclonal antibody from Amersham (Braunschweig, Germany).

Preparation of LC-Enriched Epidermal Cells Epidermal cells were prepared by the method described by Liu and Karasek [8]. Briefly, DNFB (0.5% in acetone/olive oil 4:1, Sigma, St. Louis, MO) was painted onto both sites of the ears and abdominal skin of Balb/c mice [9]. After 6 h, the ears were collected, placed in 70% ethanol for 5 sec, and rinsed with cold phosphate-buffered saline (PBS) three times. The ears were split in ventral and dorsal halves using fine tweezers and incubated separately from the abdominal skin, which was cleaned accurately from the subcutaneous tissue. Single-cell suspensions were prepared by a combined trypsin/dispase digestion according to Kitano and Okada [10]. Briefly, the ear halves and the abdominal skin were placed in PBS supplemented with 2.5 U/ml dispase type II (Boehringer Mannheim, Germany) and incubated at 37°C in PBS containing 0.2% trypsin type III (w/v) (Sigma) and 0.1% EDTA (w/v) (Serva, Heidelberg, Germany) with gentle agitation for 15 min. This procedure ensured a convenient separation of the epidermis from the dermis. The dermis was discarded while the epidermis was mechanically shredded using fine forceps. A single-cell suspension was obtained by filtering the cells through a stainless steel mesh. The residual epidermal fragments were rinsed once with RPMI 1640/10% fetal calf serum (FCS) and discarded. After passage of the resulting suspension through the mesh, both cell suspensions were combined. Cells were sedimented at 250 × *g* for 10 min at 4°C and washed twice with RPMI 1640/10% FCS.

Per mouse, 2.5×10^7 cells (viability 98%) were obtained. Bulk epidermal cells (10^6/ml) were suspended in RPMI 1640, supplemented with 10% FCS, 2 mM glutamine, 100 μg/ml penicillin/streptomycin, 50 μg/ml gentamycin, and were placed in polystyrene Petri dishes for short-term tissue culture over various periods of time. LCs were enriched to about 30% by an indirect panning protocol according to Wysocki and Sato [11].

Production of Monoclonal Antibodies The enriched LCs (5×10^6–1×10^7) were injected into DA/HA rats subcutaneously and intraperitoneally. After an immunization period of 4 months with six subsequent injections of cells, the rats were killed and the spleen was removed. The isolated lymphocytes were fused with Ag8 myeloma cells (American Type Culture Collection CRL 1580) in the presence of polyethylene glycol (PEG 4000, Boehringer Mannheim) following standard methods [12].

Subsequently, the screening of the hybridoma clones was performed by immunohistology on cryostat sections from ear skin of BALB/c mice, either untreated or treated with DNFB as described above.

Immunohistochemistry Ears from DNFB-, acetone/olive oil-treated, and untreated animals were fixed in liquid nitrogen and stored at −70°C. About 4-μm–thick cryostat sections were incubated with the MoAb 4F7 for 1 h at room temperature and subsequently incubated with a sheep–anti-rat peroxidase-conjugated polyclonal antibody for 1 h at room temperature.

The detection of specific antibody binding was achieved by the substrate 3-amino-9-ethylcarbazole (AEC). The whole method is described in detail [13]. To compare the 4F7 staining pattern with the Ia-antigen pattern, the specimens were also incubated with the MoAb MK-D6. For control purposes, the sections were incubated with normal rat serum and by omission of the 4F7 MoAb, respectively.

Immunoelectron Microscopy For immunoelectron microscopy, the ears were placed in Nakane's fixative (periodate-lysin-paraformaldehyde) for 2 h at 4°C. After being washed in 0.1 M PBS containing increasing concentrations of sucrose, the specimens were rapidly frozen in liquid nitrogen. The frozen tissue was cryostat sectioned at 40-μm intervals. The sections were incubated with the MoAb 4F7 for 2–3 h at 4°C and then incubated with peroxidase-conjugated goat–anti-rat IgG (dilution 1:25) for 4 h at 4°C. Positive reactions were visualized with 3,3-diaminobenzidinetetrahydrochloride (Sigma). After immunostaining, the sections were postfixed in half-strength Karnovsky's solution for 1 h at 4°C and 1.33% osmic acid for 1 h at room temperature, dehydrated in a graded ethanol series, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with a Philips electron microscope EM 301 using the double-condensor system. For control purposes, the tissue sections were incubated with a polyclonal rat antibody to human immunoglobulin (Ig)G or by the omission of 4F7 antibody.

Isolation of 4F7$^+$ Cells 4F7$^+$ cells were isolated by immunomagnetic beads as described by Hanau *et al* [12]. Briefly, the MoAb 4F7 was conjugated with a goat–anti-rat polyclonal antibody coupled to immunomagnetic beads and incubated overnight at 4°C on a rotor. This complex was washed three times with PBS/0.01% bovine serum albumin (BSA) to remove the unconjugated antibodies.

Dermal or epidermal cells (8×10^7) were added to the antibody complex, then incubated for 30 min at 4°C on a rotor. After 30 min, a magnetic field was applied and the non-binding cells were removed. The sediment was then washed 10–15 times with RPMI 1640/10% FCS. The cells were purified by this method to >99%, as determined by immunocytologic staining with MoAb 4F7, and subjected to ultrastructural and flow cytometric analyses.

Ultrastructural Analysis The cells were fixed in Nakane's fixative (periodate-lysin-paraformaldehyde) for 10 min at room temperature. Routine electron microscopy was performed on cell preparations that were subsequently postfixed in 1.33% osmic acid (0.05 M PBS, pH 7.4) for 20 min, dehydrated in a graded ethanol series, and embedded in Epon 812 using beam capsules. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with the electron microscope.

For immunoelectron microscopy, the cells were preincubated with 1% BSA in 0.01 M PBS and incubated with a monoclonal rat antibody against murine Ia molecules (Hybritech, San Diego, CA) for 1 h at room temperature. The antibody was solved in 0.01 M PBS containing 1% BSA. After being washed in the PBS, the cells were incubated with 10 nm gold-labeled goat–anti-rat IgG in 0.01 M PBS (1% BSA, 10% normal goat serum, 4% normal mouse serum) for 1 h at room temperature. The reaction was stopped by rinsing the cells in 0.01 M PBS. The specificity of labeling was controlled by incubating the cells with a polyclonal rat antibody against human IgG and the gold complex. After immunolabeling, the cells were processed in a manner similar to that described for routine electron microscopy.

Flow Cytofluorometric Analysis The cells (10^6/tube) were fixed with 1% paraformaldehyde in PBS without Ca^{++}/Mg^{++} ions, pH 7.1, at 20°C for 15 min under continuous gentle agitation. After washing three times in 1 ml PBS/0.1% Natriumazid/0.2% BSA, the cells were suspended in 500 μl culture supernatant of MoAb 4F7, or in a MoAb solution from the same isotype (IgG_1), for 30 min at 4°C. Following three washes with the PBS/BSA solution, 100 μl of fluorescein isothiocyanate (FITC)-conjugated goat–anti-rat IgG $F(ab')_2$, (diluted 1:200) in PBS/BSA solution were added, and the suspension was incubated for 30 min at 4°C. After three final washes, the cells were analyzed using a Becton Dickinson FACS. Per histogram, 10^5 cells were monitored.

To analyze double-stained dermal and epidermal cells, these were set up to 1×10^6 cells per 100 μl fluorescence activated cell sorter (FACS) medium per experiment, fixed for 10 min in paraformaldehyde at room temperature, and subsequently washed in FACS medium. To each cell pellet, 500 μl of an antibody suspension, mixed at equal parts with MoAbs 4F7 and MK-D6, was added. In parallel, a sample with 500 μl of isotype antibody suspension, also mixed at equal parts with anti-rat and anti-mouse antibody suspension, was set up for staining the negative and unspecific binding sites. The incubation time was also 15 min on ice, followed by three washes and uptake of the pellets in 100 μl FACS medium. Finally, the samples were mixed with each 100 μl FITC-conjugated anti-mouse IgG (Sigma), 1:200, and 100 μl phycoerythrin (PE)-conjugated anti-rat IgG, 1:100, (Southern Biotechnology Association Inc.), incubated 15 min on ice, washed three times, and then taken up in 500 μl FACS medium.

RESULTS

Immunohistochemistry The supernatants from a number of clones were screened on ear cryosections from BALB/c mice treated with 0.5% DNFB or with acetone/olive oil. A clone was selected producing an antibody weakly staining a few dendritic-appearing cells in the dermis of untreated mice and showing a strong reaction in the dermis and epidermis of DNFB-treated animals. This clone was recloned and a MoAb (4F7) was obtained. This antibody (subclass IgG_1) was used at a concentration of 10 μg/ml.

In normal skin, incubation with 4F7 MoAb resulted in staining of few dermal cells, mostly located in close apposition to the dermal blood vessels. The cells showed a dendritic-like structure (Fig 1*a*).

Six hours after application of DNFB, the staining revealed a significant increase of similar cells in the dermis and epidermis, too (Fig 1*b*). Moreover, these cells appeared slightly larger than the cells in untreated skin.

The occurrence of 4F7$^+$ cells in several organs was studied by immunohistochemistry (Fig 1 and Table I).

The 4F7 epitope was expressed on few cells in the paracortex of the lymph nodes (Fig 1*c*), the white pulp of the spleen, and the

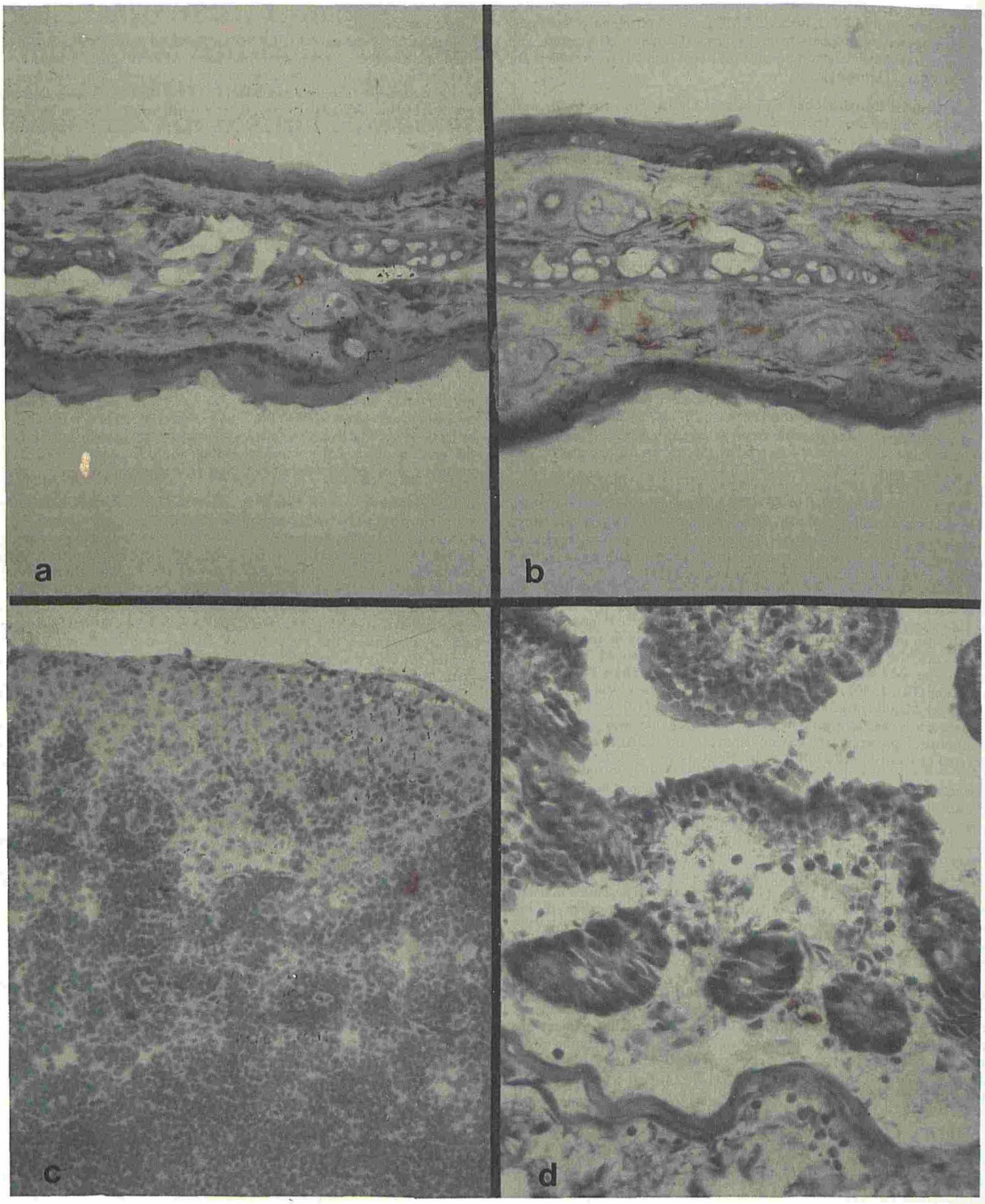

Figure 1. Immunohistochemical appearance of 4F7$^+$ cells. *a)* Ear skin of untreated Balb/c mice: positive staining of dermal cells. *b)* Ear skin of DNFB-treated Balb/c mice: staining of dermal and epidermal cells. *c)* Lymph node, untreated mice: few, positive dendritic cells in the paracortical area. *d)* Colon, untreated mice: staining of single dendritic cells in the mucosa.

Table I[a]

Pineal gland: negative
Pituitary gland: negative
Thymus gland: negative
Thyroid gland: negative
Adrenal gland: negative
Sciatic nerve: negative
Eye: negative
Colon: very few, mucosa
Lymph node: few, paracortical area
Spleen: few, white pulp and marginal zone
Peritoneal macrophages: negative

[a] The 4F7 epitope recognized a few labeled cells in the paracortical areas of the lymph nodes. The white pulp and especially the marginal zone of the spleen contained some 4F7$^+$ cells characterized by dendritic morphology. In the mucosa of the colon very few cells were recognized. In the other tissues screened, no 4F7$^+$ cells were detected.

mucosa of the colon (Fig 1*d*). All labeled cells showed a dendritic structure.

Remarkably, there was no increase of the 4F7$^+$ cells in the draining lymph nodes after DNFB application onto the ears of non-sensitized and sensitized mice.

Immunoelectron Microscopy On immunoelectron microscopy, the incubation with the 4F7 MoAb did not stain any epidermal component of normal mice. There was no labeling of keratinocytes, melanocytes, Merkel cells, and Langerhans cells.

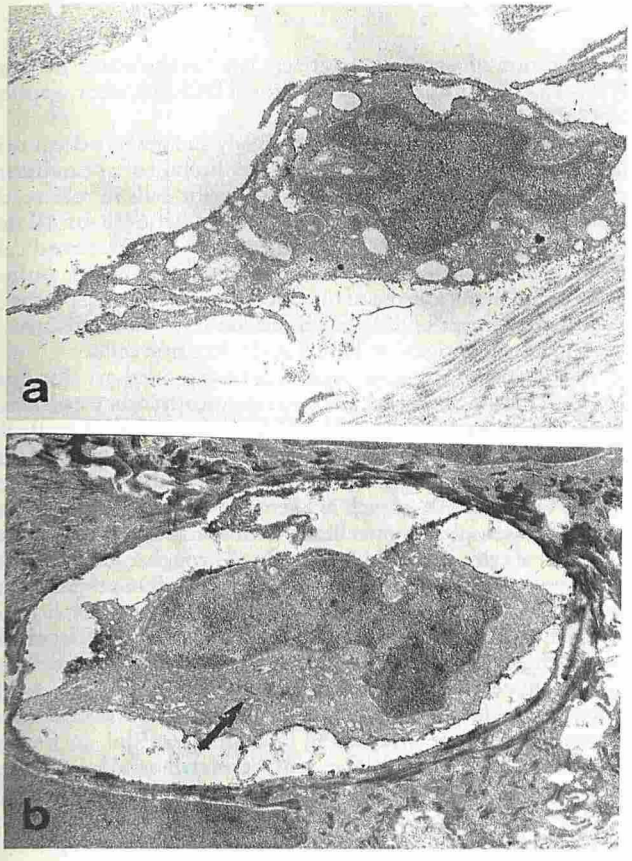

Figure 2. Immunoelectron microscopy. *a)* 4F7$^+$ dermal cell in untreated ear skin showing electron-dense peroxidase labeling of the cell membrane. Note the lobulated nucleus, the well-organized cytoplasm without Birbeck granules, and the intersectioned dendritic processes (magnification × 8500). *b)* 4F7$^+$ epidermal cell 6 h after DNFB application. The labeled dendritic cell demonstrates some cytoplasmic Birbeck granules *(arrow)* (magnification × 9500).

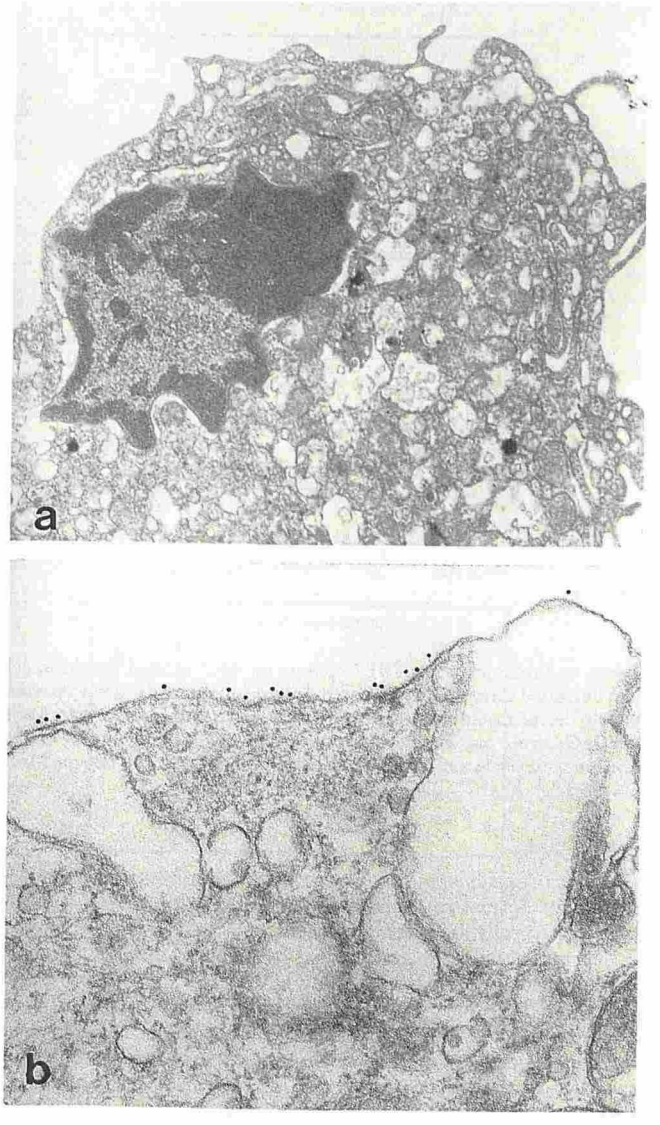

Figure 3. 4F7-enriched dermal dendritic cells on electron microscopy. *a)* Typical dendritic feature of the cells (magnification × 9500). *b)* Ia expression of the cell membrane as detected by immunogold labeling (magnification × 57,000).

The dermis regularly showed some cells with a dark peroxidase reaction product on their surface (Fig 2). These cells were characterized by a centrally located, slightly lobulated nucleus and a spindle to round-oval outer shape. The plasma membrane of the cells exhibited numerous short villous projections. The cytoplasm contained some mitochondria, smooth and rough endoplasmatic membranes, and few, if any lysosomes. The Golgi region of the cells was often prominent with electron-lucent, non-coated vesicles. No Birbeck granules were observed in the cytoplasm of the cells (Fig 2*a*).

In normal mice, only few labeled cells were found in the entire dermis. The application of 0.5% DNFB resulted in an increased number of 4F7$^+$ dermal cells and these cells often appeared larger than in the controls. A significant proportion of these activated cells were associated with the dermal capillaries. Six hours after application of DNFB, single 4F7$^+$ cells also appeared in the basal and suprabasal epidermis. On electron microscopy, these cells resembled indeterminate dendritic cells (IDCs). Moreover, about 15% of these cells showed typical Birbeck granules of LCs (Fig 2*b*). There was no ultrastructural activation (such as increased numbers of en-

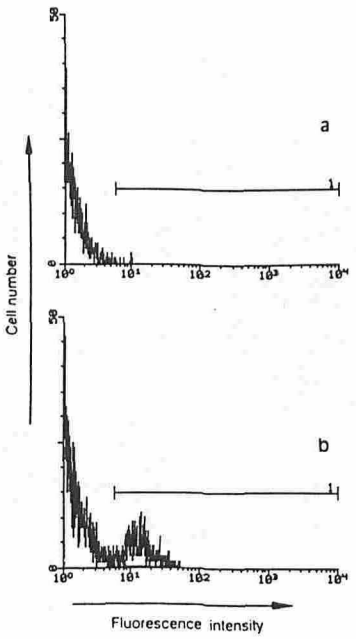

Figure 4. FACS analysis of 4F7 epitope on 4F7$^+$ dermal dendritic cells from untreated skin. Freshly prepared dermal cells were fixed with paraformaldehyde, incubated with MoAb 4F7 (IgG_1) and, as control isotype, MoAb 4C8 (IgG_1, from our laboratory), followed by FITC-labeled goat-anti-rat IgG, and were evaluated by FACS analysis. *a)* Control by an isotype MoAb 4C8 (0.10%). *b)* 4F7$^+$ dDC (1.64%).

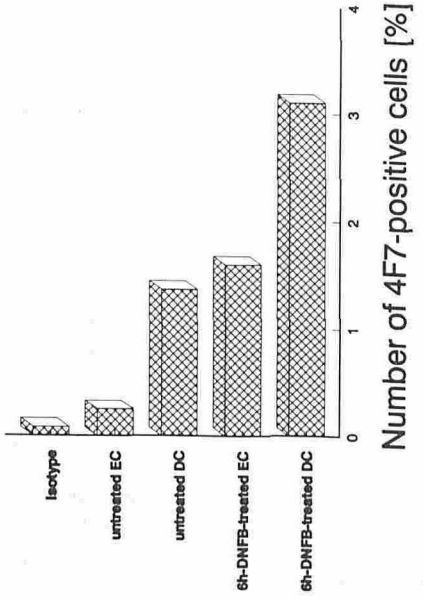

Figure 5. Upregulation of the 4F7 epitope on dermal and epidermal DCs determined in epidermal and dermal cell suspensions isolated from the ear skin of mice untreated or treated *in vivo* 6 h before with 0.5% DNFB. The mean values of three FACS experiments are shown. Standard deviation was in the range of 0.1%–0.4%. As control, the isotype MoAb 4C8 was used.

docytotic organelles, mitochondria, and endoplasmic membranes) of both the dermal and epidermal DCs, including the 4F7$^+$ LCs.

The 4F7$^+$ cells, isolated from the dermis and epidermis by 4F7-labeled magnetic beads and studied by electron microscopy, were morphologically similar to those identified *in situ* and described above (Fig 3*a*). On immunogold labeling for Ia molecules, about 50% of the dermal and about 100% of the epidermal cells were positive. A positive staining of the cell membrane was observed, especially in the larger dermal cells. These cells demonstrated a weak labeling of the plasma membrane, but did not exhibit a cytoplasmic staining (Fig 3*b*).

Flow Cytofluorometric Analysis To quantify the cells that are recognized by the MoAb 4F7, FACS analysis was performed. The cells, isolated enzymatically from dermis and epidermis of untreated and treated animals, were taken up in FACS medium, counted, and set at 10^6 cells/100 μl. As shown in Fig 4*b*, about 1.64% of the dermal cells were recognized, in contrast to the control probe with an isotype antibody (4C8 from our laboratory) that only conjugated with 0.10% of the cells (Fig 4*a*).

No 4F7$^+$ cells were detected in epidermal cells of untreated animals. In contrast, 6 h after treatment with DNFB *in vivo*, the 4F7 epitope was expressed on 3.1% of the dermal and on 1.6% of the epidermal cells (Fig 5).

The double-staining FACS analysis on cells from untreated and DNFB-treated Balb/c mice revealed that about 50% of the 4F7$^+$ dermal (Fig 6*a,b*) and 100% of the 4F7$^+$ epidermal cells express Ia molecules (Fig 6*c,d*).

DISCUSSION

To identify cell surface molecules on LCs that may be involved in the induction of allergic contact dermatitis, we have produced a MoAb against LC-enriched epidermal cells obtained from animals treated with 0.5% DNFB 6 h before. This time point was chosen because some distinct changes of the LCs, such as endocytosis [4], apposition of T lymphocytes [14], and the decrease of the ATPase activity [5] and Ia$^+$ expression [4], could already be observed 6 h after application of a contact sensitizer. We have selected an antibody that reacted with an epitope on dermal DCs and, after application of DNFB, also on epidermal DCs.

The molecule recognized by this antibody and observed on dendritic cells was upregulated after a single application of a sensitizing dose of DNFB. It was also present on dendritic cells in untreated animals, however, to a much lesser extent (about 1.64% of all dermal cells) and only on dermal dendritic cells. A few scattered cells were found in the paracortex of the lymph node, the white pulp of the spleen, and in the mucosa of the colon. Remarkably, application of a contact sensitizer onto the skin did not result in an increased expression of this antigen on lymph node dendritic cells.

In the skin, the 4F7 epitope appears to be expressed on dendritic cells only. This was shown by electron microscopy; other cells such as keratinocytes, melanocytes, fibroblasts, or endothelial cells did not express this epitope, even after application of a contact sensitizer. The cell expressing this epitope exhibited the typical ultrastructural features of DCs, such as a centrally located slightly lobulated nucleolus, a plasma membrane with numerous short villous projections, a cytoplasma with smooth and rough endoplasmatic membranes, and very few lysosomes. The 4F7$^+$ dDCs were often found in close apposition to dermal capillaries.

In addition to LCs, a number of different DCs have been described in the epidermis, dermis, and the draining lymph node, such as indeterminate, interdigitating, or veiled cells [15]. All these cells belong to a population of DCs that are bone marrow derived and in most cases have antigen-presenting functions [16,17].

Sontheimer *et al* [18,19] have recently reported on a dermal dendritic cell that is found in a preferentially perivascular localization. This dermal perivascular dendritic cell (DPDC) is Ia$^+$ and stains with several MoAbs identifying antigens of the monocyte/macrophage lineage (Leu-M5, 63D3, OKM-1), but not with the LC marker CD1 or T-, B- or NK-cell markers. In addition, its ultrastructural appearance is not consistent with mast cells or LCs. The cells are able to phagocytize 1-μm latex beads and adhere to plastic. Morphology, distribution, and data on the immunophenotype (Mohamadzadeh *et al*, submitted) indicate that the 4F7$^+$ DC is not identical to the DPDC described by Sontheimer.

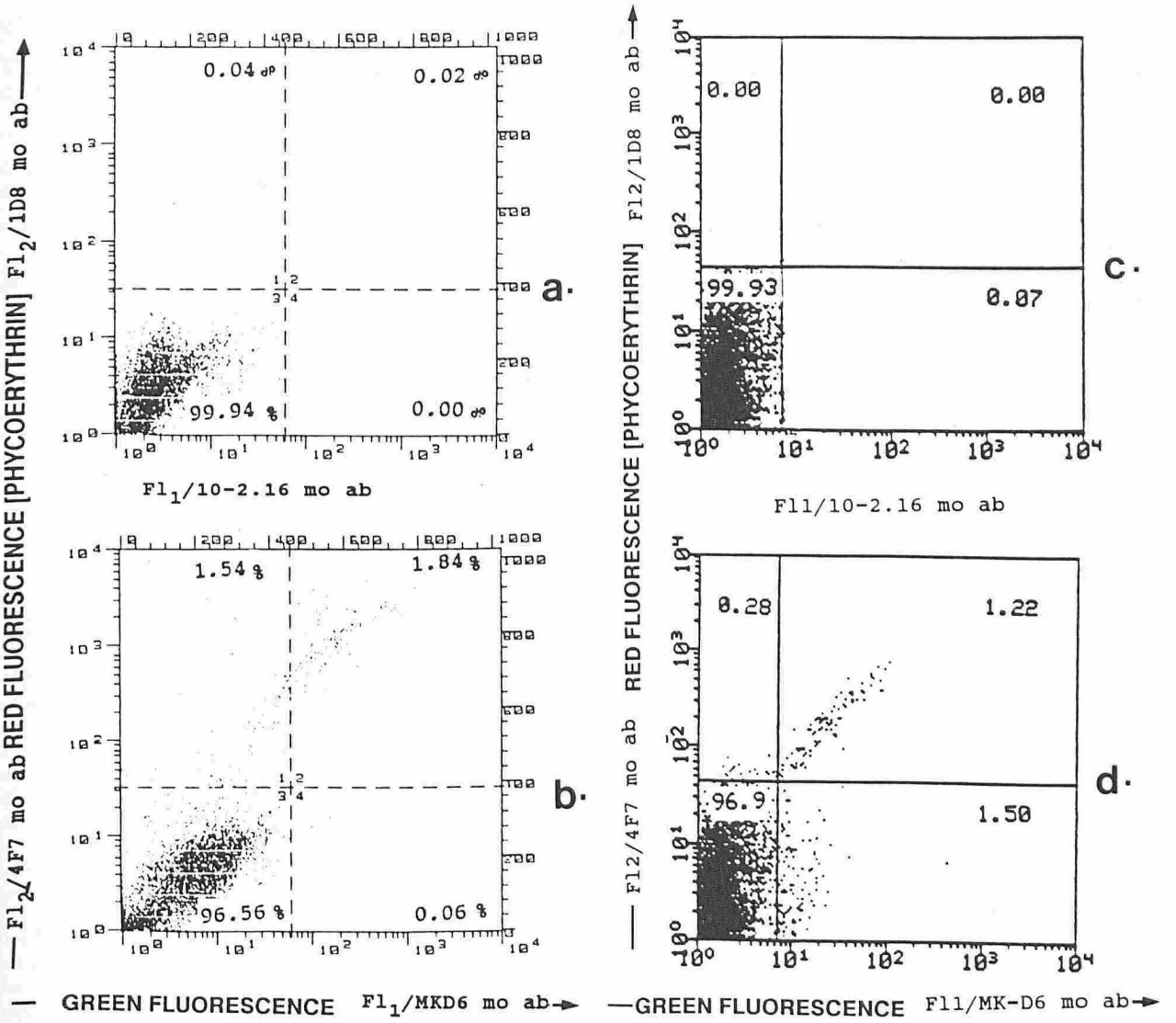

Figure 6. Expression of Ia molecules on dermal and epidermal DCs as demonstrated by FACS analysis after double-immunofluorescence labeling with the MoAbs 4F7 and MK-D6. *a,c)* Isotype antibody control (4C8 [IgG_1], 10-2.16). *b,d)* 4F7 and MK-D6 MoAbs.

As shown by FACS analysis and immunoelectron microscopy, about 50% of the $4F7^+$ dermal DCs, and 100% of the $4F7^+$ epidermal DCs express Ia molecules, indicating that these cells are capable of presenting antigen. The Ia^- dermal DCs require further analysis. It should be mentioned that culture for 3 d increases the Ia expression in these cells (Mohamadzadeh *et al,* submitted).

Morphologically, the $4F7^+$ cells do not resemble veiled cells, which have been mentioned as LCs on their way to the draining lymph node [6]. In DNFB-treated skin, the 4F7 epitope also appeared on epidermal DCs that could electron microscopically be defined as indeterminate cells (cells resembling LCs without Birbeck granules). However, 15% of the $4F7^+$ cells contained few Birbeck granules, thus identifying them as LCs. This may indicate that LCs and $4F7^+$ dermal DCs are closely related.

The exact relationship of the $4F7^+$ cell to other DCs, particularly LCs, and its exact position in the migration pathway of skin DCs remain to be analyzed. Efficient methods for the isolation and enrichment of the cell by means of this antibody have been used (Mohamadzadeh *et al,* submitted) to elucidate some phenotypical and functional properties of this cell and to define its role in contact sensitivity and other inflammatory conditions.

This work was supported by a grant of the Bundesministerium für Forschung und Technologie (BMFT 01 KC 8811).

REFERENCES

1. Marchal G, Seman N, Milon G, Truffa-Bachi P, Zilberfarb V: Local adoptive transfer of skin delayed-type hypersensitivity initiated by a single lymphocyte. *J Immunol* 129:954–958, 1982
2. Lipkow T, Saloga J, Enk A, Knop J: Zelluläre Reaktionen in der Sensibilisierungsphase des allergischen Kontaktekzems. *Allergologie* 14:176–179, 1991
3. Hanau D, Fabre M, Schmitt DA, Garaud JC, Pauly G, Tongio MM, Mayer S, Cazenave JP: Human epidermal Langerhans cells cointernalize by receptor-mediated endocytosis "nonclassical" major histocompatibility complex class I molecules (T6-antigens) and class II antigens (HLA-DR antigens). *Proc Natl Acad Sci USA* 84:2901–2905, 1987
4. Kolde G, Knop J: Epidermal Langerhans cells internalize and reexpress surface Ia molecules in allergic contact dermatitis. *Arch Dermatol Res* 283:19–20, 1991
5. Hanau D, Fabre M, Schmitt DA, Lepoittevin DP, Stampf JL, Grosshans E, Benezra C, Cazemare JP: ATPase and morphologic changes in Langerhans cells induced by epicutaneous application of a sensitizing dose of DNFB. *J Invest Dermatol* 92:689–694, 1989
6. Knight SC, Krejci J, Malkovsky M, Collizi V, Gautam A, Asherson GL: The role of dendritic cells in the initiation of immune responses to contact sensitizers. *Cell Immunol* 94:427–434, 1985

7. Kappler JW, Skidmore B, White J, Marrack P: Antigen-inducible, H-2–restricted, interleukin-2 producing T cell hybridomas: lack of independent antigen and H-2 recognition. *J Exp Med* 153:1198–1214, 1981
8. Liu SC, Karasek M: Isolation and growth of adult human epidermal keratinocytes in cell culture. *J Invest Dermatol* 71:157–162, 1978
9. Kolde G, Knop J: Ultrastructural localization of 2,4-dinitrophenyl groups in mouse epidermis following skin painting with 2,4-dinitrofluorobenzene and 2,4-dinitrothiocyanatebenzene: an immunoelectron microscopical study. *J Invest Dermatol* 90:320–324, 1988
10. Kitano Y, Okada N: Separation of the epidermal sheet by dispase. *Br J Dermatol* 108:555–560, 1983
11. Wysocki LJ, Sato VL: "Panning" for lymphocytes: a method for cell selection. *Proc Natl Acad Sci USA* 75:2844–2848, 1978
12. Goding JW: *Monoclonal Antibodies: Principles and Practice. Production and application of monoclonal antibodies in cell biology, biochemistry and immunology, 2nd ed.* Academic Press, San Diego, 1986
13. Bullock GR, Petrusz P: *Techniques in Immunocytochemistry, Volume 1.* Academic Press, London, San Diego, 1982
14. Kolde G, Knop J: Effects of immunological responsiveness on the LC behavior in experimental contact dermatitis (abstr). *J Invest Dermatol* 92:463, 1989
15. Mac Pherson GG: Lymphoid dendritic cells: their life history and roles in immune responses. Res Immunol 140:877–926, 1989
16. Katz SI, Tamaki K, Sachs DH: Epidermal Langerhans cells are derived from cells originating in bone marrow. *Nature* 282:324–326, 1979
17. Bowers WE, Berkowitz M, La Badie JH, Klinkert WEF: Differentiation of dendritic cells from bone marrow. *Transplantation Proc* 15:1611–1612, 1983
18. Sontheimer RD, Matsubara T, Seelig LL: A macrophage phenotype for a constitutive, class II antigen-expressing, human dermal perivascular dendritic cell. *J Invest Dermatol* 93:154–159, 1989
19. Sontheimer RD: Perivascular dendritic macrophages as immunobiological constituents of the human dermal microvascular unit. *J Invest Dermatol* 93:96–101, 1989